into another 1-cm cell, add 3.0 mL of the *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—This order of addition is to be followed.] At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for not less than 5 minutes. Repeat the procedure on the same dilution at least once. Absolute absorbance values are of less importance than the constancy of the rate of change of absorbance. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. The duplicate run at the same dilution should match the first run in rate of absorbance change. Determine the average absorbance change per minute, using only the values within the 3-minute portion of the curve where the rate of absorbance is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions specified in this test. Calculate the number of USP Chymotrypsin Units per mg of Crystallized Trypsin taken by the formula:

$$(A_2 - A_1) / (0.0075TW)$$

in which A_2 is the absorbance straight-line initial reading, A_1 is the absorbance straight-line final reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbance. Not more than 50 USP Chymotrypsin Units per 2500 USP Trypsin Units is found, indicating the presence of not more than approximately 5% of chymotrypsin.

Assay—

0.067 M Phosphate buffer, pH 7.6—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 13 mL of the monobasic potassium phosphate solution with 87 mL of the anhydrous dibasic sodium phosphate solution.

Substrate solution—Dissolve 85.7 mg of N-benzoyl-L-arginine ethyl ester hydrochloride, suitable for use in assaying Crystallized Trypsin (see NOTE), in water to make 100 mL. Dilute 10 mL of this solution with *0.067 M Phosphate buffer, pH 7.6* to 100 mL. Determine the absorbance of this solution, in a 1-cm cell, at 253 nm, in a suitable spectrophotometer equipped with thermospacers to maintain a temperature of 25 ± 0.1°, using water as the blank. By the addition of *0.067 M Phosphate buffer, pH 7.6*, or of the *Substrate solution* before dilution, adjust the absorbance so that it measures not less than 0.575 and not more than 0.585. Use this *Substrate solution* within 2 hours.

Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 N hydrochloric acid to obtain a solution containing about 50 to 60 USP Trypsin Units per mL.

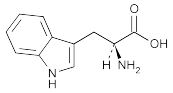
Procedure—Pipet 200 µL of 0.0010 N hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in a spectrophotometer, and adjust the instrument so that the absorbance reads 0.050 at 253 nm. Pipet 200 µL of *Crystallized Trypsin solution*, containing 10 to 12 USP Trypsin Units, into another 1-cm cell, add 3.0 mL of *Substrate solution*, and place the cell in the spectrophotometer. At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for 5 minutes. Repeat the procedure on the same dilution at least once. Plot a curve of absorbance against time, and use only those values that form a straight line to determine the activity of the Crystallized Trypsin. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. One USP Trypsin Unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified in this *Assay.*

Calculate the number of USP Trypsin Units per mg taken by the formula:

$$(A_1 - A_2) / (0.003TW)$$

in which A_1 is the absorbance straight-line final reading, A_2 is the absorbance straight-line initial reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbances.

Tryptophan



$C_{11}H_{12}N_2O_2$ 204.23

L-Tryptophan [73-22-3].

DEFINITION

Tryptophan contains NLT 98.5% and NMT 101.5% of $C_{11}H_{12}N_2O_2$, as L-tryptophan, calculated on the dried basis.

IDENTIFICATION

- **INFRARED ABSORPTION** ⟨**197K**⟩

ASSAY

- **PROCEDURE**

Sample solution: Place 200 mg of Tryptophan in a 125-mL flask. Dissolve in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid.

Analysis: Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩**:** A 0.73-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.05%). [NOTE—Gently heat the sample preparation to dissolve, if necessary.]
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:** A 0.33-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.03%). [NOTE—Gently heat the sample preparation to dissolve, if necessary.]
- **IRON** ⟨**241**⟩**:** NMT 30 ppm
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 15 ppm

Organic Impurities

- **PROCEDURE 1**

Solution A: Trifluoroacetic acid in water (1 mL/L)

Solution B: Trifluoroacetic acid in an acetonitrile and water solution (80:20) (1 mL/L trifluoroacetic acid solution)

Standard solution: 1.0 mg/L each of USP Tryptophan Related Compound A RS and USP Tryptophan Related Compound B RS in water

Sample solution: 10.0 mg/mL of tryptophan in water

System suitability solution: 1.0 mg/L of USP Tryptophan Related Compound B RS in water

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
37	35	65

Time (min)	Solution A (%)	Solution B (%)
42	0	100
47	0	100
50	95	5
60	95	5

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirement
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unspecified impurity in the portion of Tryptophan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each unspecified impurity in the *Sample solution*
r_S = peak area of tryptophan related compound B in the *Standard solution*
C_S = concentration of USP Tryptophan Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Tryptophan in the *Sample solution* (µg/mL)

Acceptance criteria
Total impurities 1: NMT 0.01% of the total impurities eluting prior to the tryptophan peak
Total impurities 2: NMT 0.03% of the total impurities eluting after the tryptophan peak. [NOTE—Exclude the peak for tryptophan related compound B.]
Tryptophan related compound A: If a peak for tryptophan related compound A is observed in the *Sample solution*, then perform the test for *Procedure 2: Limit of Tryptophan Related Compound A*, below.

- **PROCEDURE 2: LIMIT OF TRYPTOPHAN RELATED COMPOUND A**

Solution A: 18 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (9:1)
Solution B: 10 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (1:1)
Solution C: Acetonitrile in water (7:3)
Standard solution: 0.1 mg/L of USP Tryptophan Related Compound A RS in water
Sample solution: 10.0 mg/mL of Tryptophan in water
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
30	44	56	0
30.1	0	0	100
45	0	0	100
45.1	100	0	0
60	100	0	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 216 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirement
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tryptophan related compound A in the portion of Tryptophan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of tryptophan related compound A in the *Sample solution*
r_S = peak area of tryptophan related compound A in the *Standard solution*
C_S = concentration of USP Tryptophan Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Tryptophan in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 10 ppm

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –29.4° to –32.8°

Sample solution: 10 mg/mL, in water. [NOTE—Heat gently to dissolve, if necessary.]

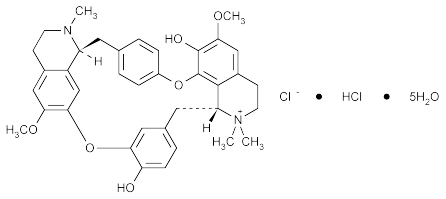
- **pH** ⟨**791**⟩**:** 5.5–7.0, in a solution (1 in 100)
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP L-Tryptophan RS
USP Tryptophan Related Compound A RS
3,3′-[Ethylidenebis(1*H*-indole-1,3-diyl)]bis[2*S*)-2-aminopropanoic]acid.
$C_{24}H_{26}N_4O_4$ 432.49
USP Tryptophan Related Compound B RS
2-Acetamido-3-(1*H*-indol-3-yl)propanoic acid.
$C_{13}H_{14}N_2O_3$ 246.3

Tubocurarine Chloride



$C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$ 771.72
Tubocuraranium, 7′,12′-dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethylchloride, hydrochloride, pentahydrate.
(+)-Tubocurarine chloride hydrochloride pentahydrate [6989-98-6].
Anhydrous 681.66 [57-94-3].

» Tubocurarine Chloride contains not less than 95.0 percent and not more than 105.0 percent of $C_{37}H_{41}ClN_2O_6 \cdot HCl$, calculated on the anhydrous basis.